

DEPARTMENT OF HEALTH AND HUMAN SERVICES

MEMORANDUM OF CONFERENCE

September 19, 1994

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Subject: Delayed ripening tomato.

Keywords: Tomato; *Lycopersicon esculentum*; delayed ripening; ACC deaminase (ACCd) from *Pseudomonas chloraphis* strain 6G5; *kan^r*; APH(3')II; Npt II.

This meeting was intended to bring Monsanto's consultation with FDA on the food and feed safety of this product to closure.

Intended Effect and Food/Feed Use

The intended effect of this genetic modification is to delay the ripening of fruit from tomato (*Lycopersicon esculentum*) plants. Tomato fruits are primarily used for human food; animal feed use is minor and is limited to occasional seasonal use.

Mechanism of Intended Effect

Ethylene serves as an endogenous phytohormone in tomatoes where it initiates and controls the rate of fruit ripening. 1-aminocyclopropane-1-carboxylic acid (ACC) is an intermediate in ethylene's biosynthetic pathway. Monsanto has isolated a gene from the common soil bacterium *Pseudomonas chloraphis* which encodes an ACC deaminase (hereafter referred to as ACCd). Expression of ACCd in tomatoes results in reduced levels of ACC and ethylene and confers a delayed ripening phenotype.

Molecular Alterations and Characterization

A restriction map of the binary vector used for *Agrobacterium*-mediated transformation of tomato plants is shown on page 15 of Monsanto's submission of August 26, 1994. Based on restriction mapping of genomic DNA from the final transgenic lines intended for commercialization, Monsanto has concluded that the inserted DNA spans the region between breakpoints which lie within a few hundred base pairs of the right and left borders of the T-DNA (refer to the Figure on page 15 of the submission). According to Monsanto, the inserted DNA is capable of expressing two proteins; 1) ACCd; and 2) the APH(3')II (Npt II) enzyme. Because APH(3')II has been approved by the agency for this intended use in tomatoes (21 CFR 173.170 and 573.130), this memorandum will not further address aspects specific to APH(3')II. Monsanto stated that sequences outside of the right and left T-DNA borders in the binary vector (specifically, the spectinomycin/streptomycin resistance gene and the pUC *ori*) were not present in tomato lines intended for commercialization as judged by Southern analysis.

Based on genomic restriction mapping and genetic analysis, Monsanto has concluded that the ACCd-expressing transgene is present in one copy, is integrated at a single locus, segregates as a single dominant Mendelian trait, and is molecularly stable over several generations. Monsanto also stated that the trait is phenotypically stable over several generations.

Expressed Protein

As stated above, the only protein expected to be expressed in Monsanto's delayed ripening tomato lines which has not previously been considered by the agency is ACCd.

ACCd catalyzes the deamination of ACC to alpha-ketobutyrate and ammonia, both of which are common metabolic intermediates in plants and other organisms. ACCd was reported by Monsanto to be inactive against amino acids other than ACC and to be heat-labile. Monsanto stated that ACCd activity has been demonstrated to be widespread

in common yeasts and bacteria. Monsanto has inferred similarity in primary sequence among these various ACCd enzymes based on the observed cross-reactivity of many with polyclonal antisera raised against ACCd from *Pseudomonas chloraphis*. According to Monsanto, ACCd is not significantly homologous in primary structure to any known toxins or allergens. Monsanto also stated that ACCd does not fit the profile of the typical allergen because 1) it is not heat stable; 2) it is not a major protein in tomatoes; and 3) it is not resistant to digestion.

In order to produce sufficient material for safety and metabolism studies, Monsanto has produced ACCd in *E. coli*. Based on comparison of molecular weight, N-terminal sequence, specific activity, immunoreactivity, and lack of glycosylation, Monsanto has concluded that *E. coli*-produced ACCd is equivalent to ACCd purified from tomatoes. According to Monsanto, ACCd is rapidly digested in simulated gastric fluid and, as expected, showed no acute toxicity in a mouse gavage study.

Compositional Analysis

Based on the nature of the genetic modification, it was not expected that delayed-ripening tomatoes would differ significantly in composition from other tomato varieties. To confirm this expectation, Monsanto carried out a compositional analysis on whole fruits.

Based on their analysis of whole fruit, Monsanto has concluded that their delayed-ripening tomatoes are not significantly different from other tomato varieties in total solids, ash, fat, total protein, carbohydrates, vitamins A, C, and B6, folic acid, riboflavin, thiamin, niacin, calcium, magnesium, iron, sodium, phosphorus, fructose, glucose, sucrose, citric acid, malic acid, lactic acid, natural tomato soluble solids, pH, titratable acidity, lycopene, and tomatine content.

Wholesomeness Studies

Monsanto described the results of a wholesomeness study they carried out in rats. On the basis of their consideration of the results of this study, Monsanto has concluded that there is no significant difference in the wholesomeness of delayed-ripening and control lines of tomatoes, as expected from their compositional analysis.

Conclusions

Monsanto has concluded, in essence, that the delayed-ripening tomato varieties they have developed are not significantly altered within the meaning of 21 CFR 170.30(f)(2) when

compared to tomato varieties with a history of safe use. At this time, based on Monsanto's description of its data and analysis, the agency considers Monsanto's consultation on this product to be complete.

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